797-Pos
A Single Actin Filament Works as a Mechanosensor
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Force plays a variety of roles in cell biology, including cell signal pathways for cell survival, growth, development, and migration. Cells may use molecular mechanisms other than mechanosensitive ion channels such as cytoskeletons to sense force, since intracellular and extracellular mechanical environments greatly affect the disassembly of stress fibers, suggesting that the tension is sensed by unknown, intracellular mechanisms and the stress fibers are disassembled. Here, we report that loss of tension in stress fibers induced disassembly of stress fibers in permeabilized semi-intact cells, which was dependent on mouse brain derived cytosolic factor(s) in the bath solution, and purified Cofilin/ADF, a major actin modulating protein ubiquitously distributed in eukaryotes, was sufficient for this stress fiber disassembly. We hypothesize that the tension decline is sensed by the actin filament, which allows cofilin to sever the filament. To test this, we have prepared a single actin filament tensed with optical tweezers and examined the tension dependent severing of the filament by cofilin. The time to sever the filament by cofilin was prolonged about 2 times in the presence of mechanical tension in the filament compared with that of a filament that was not tensed. These results indicate that tension prevents binding of cofilin to actin filaments, or prevents severing of actin filaments by cofilin. We directly observed binding of Alexa488-labeled-cofilin to actin filaments tethered on the surface of cover slip via NEM-myosin by TIRF microscopy. Scratching the ''motion restricted'' tethered actin filaments by a fine tip of glass capillaries created freely moving filament ends. The rate of binding of cofilin to the actin filaments near (<2 μm) the scratching was significantly increased. These results strongly support the hypothesis that the actin filament acts as a ''tension-sensor'' and changes susceptibility of cofilin to the filament.

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The Kinetics of Cooperative Cofilin Binding to Actin Filaments
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The interaction of cofilin with actin filaments displays positive cooperativity. The equilibrium binding and associated thermodynamic parameters of this interaction are well described by a simplified, one-dimensional Ising model with nearest neighbor interactions [De La Cruz (2005) *J. Mol. Biol. 346*, 557-564]. Here we evaluate the ability of the model to account for cooperative association kinetics and to determine the kinetic contributions to cooperative binding. A Monte Carlo based simulation protocol that allows for nearest-neighbor interactions between adjacent binding sites was employed to globally fit time courses of cofilin binding. A consistent set of binding parameters in good agreement with the equilibrium thermodynamic parameters describes well the experimental data across a wide range of cofilin concentrations. We conclude that despite its simplicity, the one-dimensional Ising model with nearest neighbor cooperative interactions, is a reliable model for analyzing and interpreting the thermodynamics and kinetics of cooperative cofilin-actin filament interactions. The methods developed for this system will be applicable to the kinetics analysis of cooperative ligand binding to linear biological polymers.

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Molecular Interaction of Cofilin with F-Actin and Implications for Filament Severing
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The regulation of actin polymerization within the cell is critical for many cell functions. Cofilin plays an important part in this process since it binds and severs actin filaments leading to depolymerization as well as the creation of new barbed ends. Although the details of cofilin's interaction with G-actin have been elucidated through a range of experimental studies, the specific interactions with F-actin have remained more elusive. Here we present the results of a detailed computational study involving a combination of protein-protein docking and molecular dynamics simulations. The resulting structural model for the cofilin/F-actin complex matches very well with existing cryoEM and mutagenesis data. Further insight is gained from a sequence and secondary structure alignment of cofilin analogs that strongly supports our binding model. Based on our binding models, we have performed simulations of sparsely and fully decorated filaments in order to gain insight into the mechanism of F-actin twisting and severing. The implications of this binding model in the function and severing action of cofilin are discussed.

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Single-Molecule Study of Actin Filament Severing by Gelsolin using Total Internal Reflection Fluorescence Microscopy
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Gelsolin-family proteins are regulators of actin polymerization dynamics.[1] To understand the mechanism of actin filament (F-actin) severing by gelsolin at the single-molecule level an *in vitro* total internal reflection fluorescence (TIRF) microscopy[2] assay has been developed. Preliminary TIRF data confirm that gelsolin sever F-actin.[3] These real-time microscopic observations are in concert with the structural and biochemical studies that have established activation of gelsolin by calcium ions. [3],[4],[5] To further ascertain a) if gelsolin preferentially binds to F-actin rather than sequestering actin monomers (G-actin), b) if it preferentially binds and severs ADP-F-actin or ATP-F-actin, and c) if gelsolin or cofilin, a member of actin depolymerizing factor (ADF), sever gelsolin-capped F-actin better than native filaments, dual color TIRFM assays are undertaken. Results will be discussed in the context of structural and biochemical data on gelsolin-family of proteins.
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801-Pos
Measurement of Filamin a Torsion In-Singulo
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Filamin A (FLNa) is one of the most ubiquitous actin binding proteins that cross-link actin filaments. The protein is a v-shaped homodimer. Each monomer consists of twenty-four β-sheet ''rod-like'' domains which separate the actin binding domain at the N-terminus from the C-terminus at the dimerization site. The rod-like domains are divided by two unstructured ''hinge'' sequences between repeats 15 and 16 and repeats 23 and 24. While it has been reported that these hinge sequences play a key role in determining the nonlinear elastic response of stressed actin networks, how the hinge segment elicits such changes in network elasticity remains unknown. To elucidate this mechanism, we measure the torsional dynamics of single FLNa molecules in real-time by using high-resolution, fluorescence microscopy. Using a native-like single-molecule assay consisting of the complex formed by a surface-bound actin filament cross-linked to a freely-rotating actin filament by FLNa we are able to track the instantaneous angle between the cross-linked filaments as the cross-linked filament undergoes a thermally-driven rotational motion. By estimating the torsional stiffness of both wildtype and hingeless FLNa isoforms, the role of the hinge sequence in determining the torsional rigidity of FLNa is inferred. Both hinged and hingeless FLNa form cross-links with comparable near-acute angles. Moreover, the torsional dynamics of each cross-link can be distinctly categorized by using this strategy to image the molecular interactions present in each cross-link. This work may illuminate the molecular origins of the nonlinear elasticity observed in FLNa-cross-linked F-actin networks. Support from the Singapore-MIT Alliance for Research and Technology and the NSF Career Award (0643745) are gratefully acknowledged.

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Actin Crosslinking Proteins Recognize Distinct Arrangements of Actin Filaments
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Self-assembly of complex structures is commonplace in biology but often poorly understood. In the case of the actin cytoskeleton, a great deal is known about the components that comprise higher order structures, such as lamellar meshes, filopodial bundles and stress fibers. Each of these cytoskeletal structures contains actin filaments and crosslinking proteins, but the role of crosslinking proteins in the initial steps of structure formation has not been clearly elucidated. We employ a novel optical trapping assay to investigate the behaviors of fascin and alpha-actinin during the first steps of structure assembly. Here we show that these proteins have distinct binding characteristics that cause them to recognize and crosslink filaments that are arranged with specific geometries. Alpha-actinin is a promiscuous crosslinker, linking filaments over a range of angles. These angles include parallel (0^0), anti-parallel (180^0), and series of intermediate crossing angles ($15\text{-}165^0$). It is also an extremely flexible crosslinker, maintaining connection even when the link is rotated. Conversely, fascin is extremely selective, only crosslinking filaments in a parallel